Characterisation of the functional consequences of PTEN gene mutations in colon cancer.

By

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STATEMENT OF ORIGINALITY

I certify that the work of this thesis has not been submitted for a degree nor has been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that thesis has been written by me. Any help I have received in my research work and preparation of the thesis has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

JIGNA BHATIA SHARMA.

THIS THESIS WORK IS DEDICATED TO MY GRANDMOTHER MRS BHAGIRATHI V BHATIA

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LIST OF ABBREVIATIONS

Amp	Ampicilin
APC	Adenomatous polyposis coli
AS	Antisense
AIP	Apoptosis inhibitory factor
AKT	AKT/protein kinase B
ASK	Apoptosis signal regulated kinase
ATP	Adenosine triphosphate
β (B) actin	Beta actin
BAD	Bcl-2 associated death promotor
b.p	Base pairs
BSA	Bovine Serum Albumin
Ca2+	Calcium ion
Caspase	Cystein aspartate specific proteases
CBF1	CBF1 also known as recombination signal binding protein for immunoglobulin kappa J region (RBBJ)
CENP-C	Centromere protein C
cGMP	Cyclic guanosine monophosphate
CK	Creatine kinase
CK2	Casein kinase II
CMV	Cytomegalovirus
CO2	Carbon dioxide
cDNA	Complimentary DNA
CRC	Colorectal Cancer
CDK	Cyclin dependent kinase
DEPC	Diethyl pyrocarbonate
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
dNTPs	deoxynucleotide triphophates
Dest	Destination
DNA	Deoxyribonucleic acid

DTT	Dithiothreitol
ECL	Electrochemiluminescence
EDTA	Ethylenediamminetetraacetic acid
ELISA	Enzyme linked immunosorbant assay
eNOS	Endothelial nitric oxide synthase
Entr	Entry
EtBr	Ethidium bromide
FasL	Fas ligand
FBS	Foetal bovine serum
FCS	Foetal calf serum
FLASH	4', 5'-bis (1, 3, 2-dithioarsolan-2-yl) fluorescein
G	Grams
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GSK3b	Glycogen synthase kinase 3b
GW	Gateway
HEPES	N-2-hydroxyethlpiperazine-N'-ethanesulphonic acid
HIF1a	Hypoxia inducible factor 1a
HNPCC	Hereditary non-polyposis colorectal cancer
HRP	Horse radish peroxidase
HSP	Heat shock protein
IGF1	Insulin like growth factor
IPTG	Isopropyl-B-D thiogalactopyranoside
kb	Kilobase
kda	KiloDalton
LB	Luria-Bertani broth
LOH	Loss of Heterozygosity
MAGI3	Membrane-associated guanylate kinase with inverted orientation
MAPK	Mitogen activated protein kinase
Mdm2	Murine double minute 2
MMR	Mismatch repair
mRNA	Messenger RNA
MSI	Microsatellite instability
MSI+	Microsatellite unstable (tumour)
mTOR	Murine target of rapamycin

MSI-	Microsatellite stable (tumour)
NADPH	Nicotinamide adenine dinucleotide phosphate
NEDD4-1	Neural precurser cell expressed developmental down Regulated 4-1
NFκB	Nuclear factor kappa light chain enhancer of activated B cells
p42/p44 (MAPK)	Protein 42/ protein 44 mitogen activated kinase
p70S6K	70 kDa ribosomal protein S6 kinase
PARP	Poly (ADP-ribose) polymerase
PCAF	p300/CBP associated factor
PDGF	Platelet derived growth factor
PDK1	phosphoinositide dependent protein kinase1
PDZ	Post synaptic density protein, Drosophila disc large tumour suppressor and Zonula occludens-1 protein
PI	Propidium iodide
PINK1	PTEN induced kinase 1
PIP2	Phosphatidylinositol 2 phosphate
PIP3	Phosphatidylinositol 3 phosphate
PI3K	Phosphatidylinositol 3 kinase
PKC	Protein kinase C
PPAR	Peroxisome proliferators-activated receptors
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTP	Protein tyrosine phosphatases
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RCF	Relative centrifugal force
RNA	Ribonucleic acid
ROCK	Rho associated kinase 1
RNA	Ribonucleic acid
RT	Room temperature
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SDS PAGE	Sodium disulphide poly acrylamide gel electrophoresis
SHIP	SRC homology 2 containing inositol 5 phosphatase

SHP1	**The Src homology domain 2 (SH2) containing tyrosine phosphtase 1**
SHP2	**The Src homology domain 2 (SH2) containing tyrosine phosphtase 2**
Spect	**Spectinomycin**
Ta	**Annealing temperature**
Taq polymerase	***Thermus aquaricus* DNA polymerase enzyme**
TE	**Tris/EDTA buffer (10mM Tris, pH8; 1mM EDTA**
TBS	**Tris buffered saline**
TEMED	**Tetramethylethlenediamine**
Tm	**Melting temperature**
Vect	**Vector**
WT	**Wild type**
WB	**Western Blotting**

Conference Presentation:

1. Jigna Bhatia, Bronwyn O' Brien, Glenn Lobo, Najah Nassif. Cancer-Associated *PTEN* Mutations Alter PTEN function in colon and other cell line. 24th Annual Combined RNSH/UTS/USYD held on the 18th – 19th November 2008. (Selected in young investigators category) Oral presentation.

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3. J. Bhatia, B.A. O' Brien, G.P. Lobo, N.T. Nassif. Alteration of *Pten* Function by Cancer-Associated *Pten* Mutations. 20th National Lorne cancer conference 14th -16th February 2008. Poster presentation

4. J. Bhatia, B.A. O' Brien, G.P. Lobo, N.T. Nassif. Alteration of *Pten* Function by Cancer-Associated *Pten* Mutations. "PTEN Pathways & Targets" March 5 - 9, 2008. Poster presentation.

5. Jigna Bhatia, Bronwyn O' Brien, Glenn Lobo, Najah Nassif. Alteration of *Pten* Function by Cancer-Associated *Pten* Mutations. ASMR Medical Research Week - State/Territory Scientific Meetings at ASMR conference June 2 - 8, 2008.Oral presentation.

6. Jigna Bhatia, Bronwyn O' Brien, Glenn Lobo, Najah Nassif (2009). Cancer associated PTEN mutations alter PTEN function In colon and non-colon cancer cell lines (manuscript in preparation).

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Abstract

Colon cancer constitutes the second most common cause of cancer death in many Western countries. The *PTEN* tumour suppressor gene, located on chromosome 10q23.3, is now recognised as the most highly mutated tumour suppressor gene. PTEN, a lipid and protein phosphatase, regulates the phosphatidylinositol 3-kinase (PI3K)/ Akt signalling pathway and modulates cell cycle progression and cell survival. Previous work at University of Technology of Sydney laboratory has shown that a significant proportion of sporadic colorectal tumours harbour *PTEN* mutations that alter gene function and may therefore contribute to the pathways of colorectal carcinogenesis. A total of 10 novel somatic mutations have been described. In order to determine the functional consequences of these colon cancer-associated PTEN mutations, the wild type (WT) *PTEN* gene was cloned into a mammalian expression vector system and each of the mutants were generated from this. The WT, and each of the mutant K62R, Y65C, K125E, K125X, E150Q, D153N, D153Y, V217A 319X and N323K PTEN constructs were then transiently transfected into an U87MG glioblastoma PTEN null cell line and HCT116 colon cancer PTEN expressing cell lines that were then assayed for cell cycle phase distribution, Akt phosphorylation levels and cell proliferation. The analyses of endogenous suppression of Phospho Akt assay indicates 50% of PTEN mutants (Y65C, K125E, K125X, D153N, and 319 X) shows deficiency in the U87MG cell line and 70% of the mutants in the HCT116 cell line (Y65C, K125E, K125X, D153N, D153Y V217A and 319X) had deficiency in suppressing endogenous phosphorylated Akt. The results obtained show 50% (Y65C, K125X, K125E, D153N and 319X) of the PTEN mutants had functional deficiency in cell cycle inhibitory capacity in the S phase in the U87MG cells; in contrast 80% (Y65C, K125X, K125E, E150Q, D153N, D153Y, V217A and 319X) of the PTEN mutants had functional deficiency in cell cycle inhibitory capacity in the S phase in the HCT116 cells. The results obtained show 60% of the PTEN mutants (K62R, Y65C, K125E, K125X, D153N and 319X) had alteration in cell proliferation rate in U87MG cells. In contrast in the HCT116 cell lines, 80% of the PTEN mutants (Y65C, K125E, K125X, E150Q, D153N, D153Y, V217A and 319X) had alteration in cell proliferation rates. These three functional assays of the mutations tested show an alteration of PTEN function. This was observed as a marked reduction in the ability of these PTEN mutants to bring about a level of cycle arrest, reduction of Akt phosphorylation levels and cell proliferation, compared to that observed with the WT *PTEN* gene product. These studies reveal that *PTEN* gene somatic mutations do alter PTEN function and are therefore

likely to contribute to the process of colorectal carcinogenesis and may mediate a PTEN-associated carcinogenic pathway in these tumours.